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OLIGOMERIC PROANTHOCYANIDIN GLYCOSIDES OF *Clementsia semenovii* AND THEIR BIOLOGICAL ACTIVITY. III.

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The structures of two oligomeric proanthocyanidins isolated from roots of Clementsia semenovii *have been established using chemical and spectral data. Animal studies showed that these compounds possess hypocholesterinemic, hypolipidemic, and anti-inflammatory activities.*

Key words: oligomeric proanthocyanidins, hypocholesterinemic, hypolipidemic, and anti-inflammatory activity.

The proanthocyanidin composition of *Clementsia semenovii*, which belongs to the Grassulaceae family, have been reported [1-5]. It was previously named *Rhodiola semenovii* [6, 7]. In continuation of these studies we isolated two new proanthocyanidins: 7-O-[β-D-Glc*p*-O-β-D-Glc*p*]$_2$-3-O-galloyl-(-)-epigallocatechin-(4β-8)-[3-O-galloyl-(-)-epigallocatechin]-(4β-8)-3-O-galloyl-(-)-epigallocatechin, rhodichimoside (**1**) and 7-O-β-D-Glc*p*-3-O-galloyl-(-)-epigallocatechin-(4β-8)-[(-)-epigallocatechin]$_2$-(4β-8)-(-)-epigallocatechin-(4β-6)-3-O-galloyl-(-)-epigallocatechin, rhodichin (**2**). Compounds **1** and **2** were isolated by extraction with aqueous ethanol, concentration, dilution with water, and extraction with diethylether, ethylacetate, and butanol. The butanol extract was separated on microcrystalline cellulose with subsequent rechromatography on Sephadex LH-20.

Rhodichimoside (**1**) is an optically active amorphous powder ($[\alpha]_D^{22}$ -16°, *c* 0.32, ethanol) that decomposes at 290-300°C and has molecular weight ~2500. The UV spectrum of **1** exhibits maxima characteristic of galloyl proanthocyanidins. The IR spectrum of **1** contains absorption bands of hydroxyl, α-carbonyl of aromatic acid, aromatic rings, and an ester.

The ^{13}C NMR of **1** recorded with full H-decoupling contains signals of gallocatechins with 2,3-*cis*-configurations (signals are lacking at 80-83 ppm, which are characteristic of 2,3-*trans*-moieties). The chemical shifts of ring *C* carbons (C-2, C-3, C-4) are similar to those of galloyl epigallocatechins (**3**) in proanthocyanidins (Table 1). Table 1 shows that the signals at 153.6-155.0 ppm belong to O-substituted C atoms C-5, C-7, and C-9 of ring *A*. Ring *A* atoms C-6 and C-8, which have no interflavan bonds, appear near 97.0 ppm whereas atoms involved in interflavan bonds appear near 107.0 ppm [8].

Analysis of the chemical shifts of ring *B* C atoms showed that **1** consists of only gallocatechin units. The strong signal near 145.6 ppm belongs to substituted C-3′ and C-5′ of ring *B*. We assigned the resonance at 107.0 ppm to C-2′ and C-6′. Shielded C-4′ resonates near 133.6 ppm. The signal at 130.7 ppm belongs to C-1′ of ring *B* in gallocatechins [9, 10].

The appearance of the signals for ring *C* C-2 at 73.8 and 77.0 ppm indicates that the flavan-3-ol asymmetric centers of the proanthocyanidin have the 2,3-*cis*-configuration [10, 11]. Furthermore, the spectrum of **1** has signals for β-glucose (**4**) and gallic acid moieties. Most of the glucose signals overlap with those of C atoms in heterocyclic ring *C* of the proanthocyanidin. Thus, signals of C-4 are most useful for determining that C-3 is esterified.

Signals for C-4 of the "upper" units appear at 34.7 ppm; those of the "lower" unit, at 26.0 ppm. This unambiguously indicates that all gallocatechin units have gallic acid units. In this instance, the glucan unit exhibits signals for the sixth glucose C atom (61.8, 63.5, and 66.0 ppm).

Enzymatic cleavage of **1** and acid hydrolysis of its permethylate (**7**) demonstrated that the sugar portion in fact consists of four D-glucoses with β-glycosidic bonding to the aglycone and (6→1)-β-bonding to each other. Acid (**3-5**) and thiolytic cleavage of **1** (**3, 6**) suggests that the glucan unit is bonded to the upper unit.

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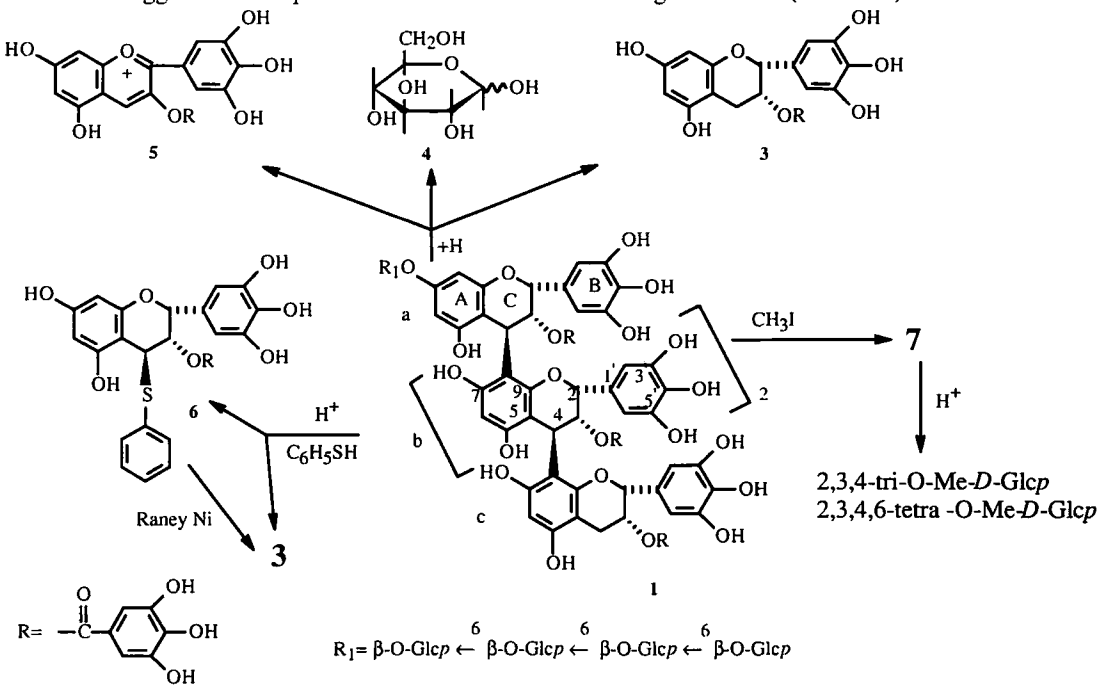
TABLE 1. ^{13}C NMR Chemical Shifts of Rhodichimoside (1), ppm, 0 = TMS (acetone—water, 1:1)

Carbon atom	Rhodichimoside fragment				
	a	b	c	glucose	galloyl
2	73.8	77.0	77.0**		
3	73.8	73.8	69.0		
4	34.7	34.7	26.0		
6	97.0	97.0	97.0		
8	97.0	97.0	107.0		
10	102.9*	102.9*	100.1*		
5, 7, 9	153.6-155.0	153.6-155.0	153.6-155.0		
1′	130.7	130.7	130.7	102.9*	121.3
2′	107.0	107.0	107.0	73.8	110.6
3′	145.6	145.6	145.6	77.0**	145.6
4′	133.6	133.6	133.6	70.4	139.0
5′	145.6	145.6	145.6	77.0**	145.6
6′	107.0	107.0	107.0	61.8; 63.5; 66.0;67.2	110.6
-COO-					169.3

*,**Signals can be inverted.

The data presented above are similar to the physicochemical and spectral properties of rhodisinoside [2]. However, rhodichimoside has a greater molecular weight than rhodisinoside. Therefore, the degree of polymerization is greater.

The results suggest the most probable structure and relative configuration of **1** (Scheme 1).

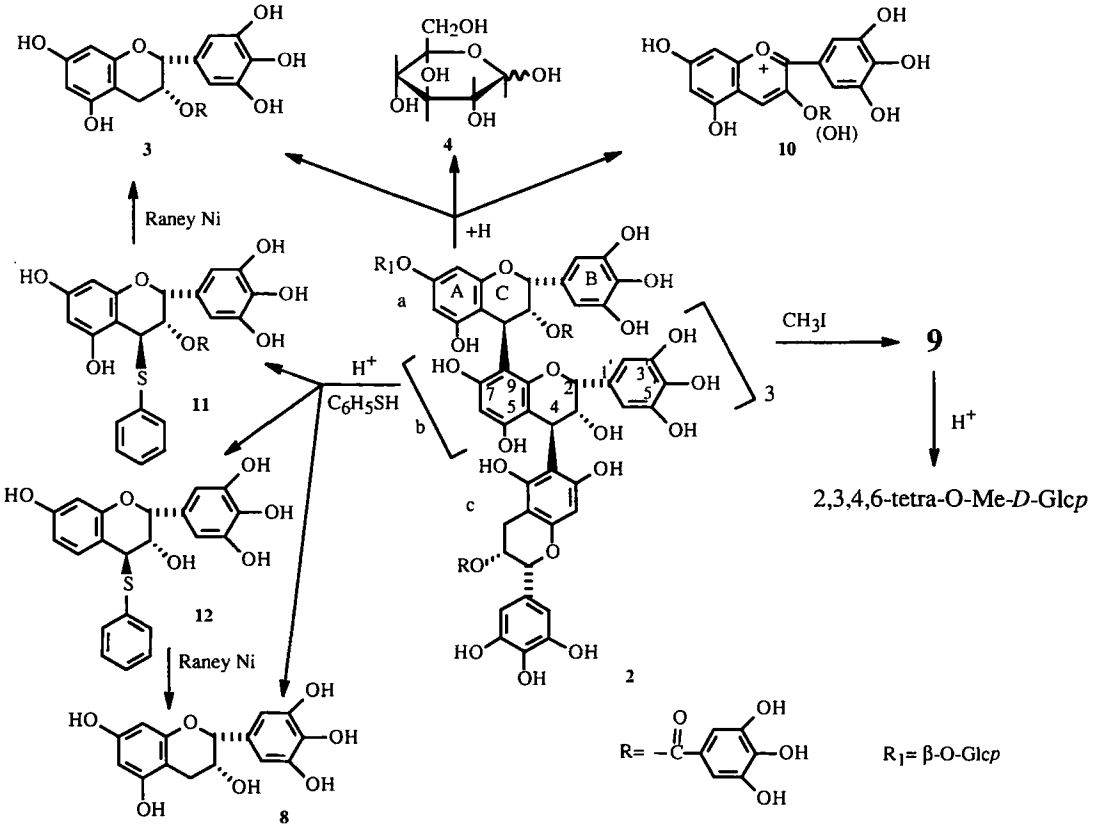


Scheme 1.

TABLE 2. ^{13}C NMR Chemical Shifts of Rhodichin (**2**), ppm, 0 = TMS (acetone—water, 2:1)

Carbon atom	Rhodichin fragment				
	a	b	c	glucose	galloyl
2	75.4	77.2**	77.2**		
3	74.0	72.3	69.3		
4	34.6	36.9	-*		
6	96.9	96.9	106.7		
8	96.9	106.7	96.9		
10	101.8**	101.8**	99.3		
5, 7, 9	157.8	157.8	157.5		
1′	130.0	130.0	130.0	101.8**	121
2′	106.7	106.7	106.7	74.2	110.3
3′	145.8	145.8	145.8	76.4	145.8
4′	132.1	132.1	132.1	70.7	138.4
5′	145.8	145.8	145.8	77.2**	145.8
6′	106.7	106.7	106.7	62.7	110.3
-COO-					166.8

*Signal not observed owing to overlap with solvent signal.
**Signals can be inverted.



Scheme 2.

TABLE 3. Effect of Rhodichimoside, Rhodichin, and Polysponin on Cholesterol and Triglyceride Content in Normal Rat Serum ($M \pm m$, $n = 8$)

Experimental conditions	Cholesterol, mmol/l	Effect, % of control	*P*	Triglycerides, mmol/l	Effect, % of control	*P*
Intact animals	1.72±0.048	-	-	0.44±0.014	-	-
		Single dose				
Rhodichimoside	1.62±0.038	-5.8	<0.25	0.42±0.012	-4.6	<0.5
Rhodichin	1.57±0.052	-8.7	<0.1	0.41±0.010	-6.8	<0.25
Polysponin	0.52±0.050	-11.6	<0.02	0.40±0.020	-9.1	<0.25
		Multiple dose				
Rhodichimoside	1.50±0.056	-12.8	<0.02	0.40±0.014	-9.1	<0.1
Rhodichin	0.46±0.054	-15.1	<0.01	0.39±0.012	-11.4	<0.02
Polysponin	1.42±0.044	-17.5	<0.001	0.38±0.012	-13.6	<0.01

According to UV and IR spectra, rhodichin (**2**) is also a proanthocyanidin with molecular weight ~2600. It decomposes at 300°C without melting and has $[\alpha]_D^{22}$ +42° (*c* 0.42, ethanol). The ^{13}C NMR of **2** contains signals of epigallocatechins, gallic acid, and glucose (Table 2).

The arrangement of the chemical shifts of ring *C* C atoms indicates that the proanthocyanidin consists of gallocatechin (**8**) units with the 2,3-*cis*-configuration and a part of the epigallocatechins in the chain have gallic acid moieties (34.6 ppm, C-4). A unit with gallic acid (**3**), 69.3 ppm for C-3 (the solvent signal overlaps the C-4 signal), also occupies the lower position in the proanthocyanidin. The interflavan bonds in rhodichin are mainly of the C-4–C-8 type. The signal at 99.3 ppm suggests that one of the interflavan bonds is of the C-4–C-6 type. The sugar signals mostly overlap those of ring *C* epigallocatechin C atoms. Only the C-6 signal occurs outside this region at 62.7 ppm.

Enzymatic cleavage and acid hydrolysis of rhodichin permethylate (**9**) demonstrated that the sugar portion consists of one D-glucose (**4**) β-bonded to the aglycone.

Acid (**3-5**, **10**) and thiolytic (**3**, **8**, **11**, **12**) cleavage of **2** indicates that the lower gallocatechin unit lacks a sugar unit. Thus, it seems that the upper epigallocatechin unit is glycosylated if the stereochemical hindrance of rhodichin is considered.

Thus, the data suggest the most probable structure and relative configuration of rhodichin **2** (Scheme 2).

Animal studies of the biological properties of rhodichimoside and rhodichin showed that these compounds are interesting primarily for their hypocholesterinemic activity. This was found not only in normal animals but also in animals with experimental hyperlipidemia. Table 3 shows that the cholesterol content in normal rat serum was significantly reduced (by 5.8-8.7%) by a single administration of these compounds. Administering rhodichimoside and rhodichin for 10 days produced a statistically significant reduction in cholesterol by 12.8-15.1%.

It is noteworthy that multiple administration of these compounds elicits a small hypolipidemic effect that, for rhodichin administration, is also significant. However, in both instances the ability to lower cholesterol levels in rat serum was less for the studied compounds than for polysponin, a known medicine for this treatment.

A study in rats with various hyperlipidemias produced results that were more significant and comparable to those with polysponin. Table 4 shows that the cholesterol and triglyceride levels in rat serum increased relative to the normals by 37.2 and 27.3%, respectively, after a 24-h fast. The levels increased by only 9.3 and 9.1% for animals that received rhodichimoside and by 6.9 and 4.5% for those receiving rhodichin (significant differences). The observed effect of the studied compounds in these experiments was comparable to and sometimes even greater than that of polysponin. The cholesterol level in the serum of rats with hyperlipidemia caused by fasting increased by 10.5%; the triglyceride level, by 9.1%, after the administration of polysponin.

The results were similar for hyperlipidemia caused by triton WR-1339. Table 4 shows that the rise of chloesterol and triglyceride levels for the control group of rats was much more evident than for that caused by fasting (3.7 and 12.9 times, respectively). However, the effect of the studied proanthocyanidin polymers in this instance was much more noticeable and very similar to that of polysponin.

TABLE 4. Effect of Rhodichimoside, Rhodichin, and Polysponin on Cholesterol and Triglyceride Content in Serum of Rats with Various Hyperlipidemic Conditions ($M \pm m$, $n = 8$)

Experimental conditions	Cholesterol, mmol/l	Effect, % of control	P, rel. to control	Triglycerides, mmol/l	Effect, % of control	P, rel. to control
Control I (hyperlipidemia caused by 24-h fast)	2.36±0.16	-	-	0.56±0.030	-	-
Rhodichimoside + 24-h fast	1.88±0.12	-20.3	<0.05	0.48±0.022	-14.3	<0.05
Rhodichin + 24-h fast	1.84±0.10	-22.1	<0.05	0.46±0.018	-17.9	<0.02
Polysponin + 24-h fast	1.90±0.08	-19.5	<0.05	0.48±0.020	-14.3	<0.05
Control II (triton WR-1339)	6.40±0.52	-	-	5.70±0.32	-	-
Rhodichimoside + triton WR-1339	4.94±0.40	-22.8	<0.05	4.76±0.28	-16.5	<0.05
Rhodichin + triton WR-1339	4.80±0.30	-25.0	<0.05	4.66±0.22	-18.3	<0.02
Polysponin + triton WR-1339	4.90±0.34	-23.4	<0.05	4.70±0.20	-17.6	<0.02

TABLE 5. Effect of Rhodichimoside, Rhodichin, and Cortisone Acetate on Formalin, Dextran, and Serotonin Adema of Rat Feet ($M \pm m$, n = 8-10)

Experimental conditions	Rat-foot swelling relative to initial, ml	Swelling inhibition, %	P
3 h after formalin administration			
Control	0.47±0.036	-	
Rhodichimoside	0.33±0.034	29.8	<0.02
Rhodichin	0.31±0.030	34.1	<0.01
Cortisone acetate	0.30±0.028	36.2	<0.002
2 h after dextran administration			
Control	0.66±0.044	-	
Rhodichimoside	0.51±0.038	22.7	<0.02
Rhodichin	0.49±0.034	25.8	<0.01
Cortisone acetate	0.48±0.024	27.3	<0.002
2 h after serotonin administration			
Control	0.70±0.058	-	
Rhodichimoside	0.50±0.050	28.6	<0.02
Rhodichin	0.48±0.048	31.4	<0.01
Cortisone acetate	0.47±0.046	32.9	<0.01

Thus, whereas for rats receiving rhodichimoside and rhodichin the cholesterol level in serum after intraperitoneal administration of triton WR-1339 increased by 2.87 and 2.79 times (22.8 and 25.0% lower than the control) and the triglyceride level, by 10.82 and 10.59 times (16.5 and 18.3% less than the control); for those receiving polysponin the increase of cholesterol was 2.84 times and of triglyerides, 10.68 times (23.4 and 17.5%, respectively, of the control).

The encouraging results concerning the hypolipidemic activity of rhodichimoside and rhodichin are even more significant because these compounds also exhibit rather good anti-inflammatory activity. Rhodichimoside and rhodichin were active in rats with various types of inflammation and were only slightly inferior to cortisone acetate (Table 5).

The abilities of rhodichimoside and rhodichin to decrease swelling caused by formalin were 29.8 and 34.1% (cortisone acetate, 36.2%). The results were similar if other phlogogenic agents were used. The studied compounds decreased dextran swelling by 22.7-25.8%; serotonin, by 28.6-31.4%. Cortisone acetate in these instances gave 27.3 and 32.9%. Table 5 shows that all results are statistically valid.

The data indicate that preparations for treating various pathological conditions accompanying hypercholesterinemia, hyperlipidemia, and inflammation that are based on rhodichimoside and rhodichin show great promise. The hypolipidemic, anti-inflammatory, and antihypoxic activity [12] of these compounds suggest that they should be studied as potential

antiatherosclerotic agents [13], the role of which in the treatment and cure of certain cardiovascular pathologies has substantially increased in recent years.

EXPERIMENTAL

General Observations. UV spectra of proanthocyanidins and their derivatives were recorded in alcohol on a Hitachi (Japan) EPS-3T instrument. IR spectra were recorded on a Karl-Zeiss-Jena (Germany) dual-beam UR-20 spectrophotometer in the range 3600-750 cm^{-1} in KBr pellets. ^{13}C NMR spectra were recorded on a TESLA (Czech Republic) BS 567 A/25 MHz instrument in deuteroacetone and a deuteroacetone:deuterowater (1:1, 2:1) mixture. Tetramethylsilane ($\delta = 0$) was used as an internal standard. Concentrations varied in the range 15-20%.

Molecular weights were determined on a MOM 3170 (Hungary) ultracentrifuge at 8000 rpm, 20°C, 30° angle, and 30 min duration. The distribution of average molecular weights was found by gel filtration on a Sephadex LH-20 column.

The optical activity of the proanthocyanidins was determined using a JACSO J-20 instrument.

The proanthocyanidins were separated by column chromatography using LK microcrystalline cellulose powder (Czech Republic). The compounds were identified and their purity determined using TLC on Silufol UV-254 (Czech Republic) plates and the following solvent systems: 1) $CHCl_3$—ethylacetate (1:2-4); 2) $CHCl_3$—CH_3OH—ethylacetate (10:1.5:1), (5.5:1.5:1); 3) $CHCl_3$—acetone—formic acid—water (5:10:8:3); 4) hexane—ethylacetate (2:1); 5) $CHCl_3$—acetone (3.5:10). The plate length was 15 cm. Spots were detected visually using 1% vanillin in 5% H_2SO_4 and a mixture (1:1) of 1% aqueous solutions of $FeCl_3$ and $K_3[Fe(CN)_6]$.

The isolated compounds were dried in a drying pistol over anhydrous $CaCl_2$ at 60-80°C.

Analytical data corresponded to those calculated.

Extraction and Isolation of Proanthocyanidins. Ground air-dried roots of Semenov rodiol (4 kg) were extracted six times wiht 96% ethanol (once with 24 l and five times with 20 l). The extracts were combined and evaporated under vacuum at 40-50°C and 30 mm Hg until the volume was 2 l. The condensed extract was treated successively with diethylether (500 ml, five times) to remove low-molecular-weight slightly polar compounds, ethylacetate (500 ml, five times) to remove slightly polymerized proanthocyanidins and their glycosides, and *n*-butanol (500 ml, five times) to isolate moderately polymerized proanthocyanidins and glycosides. The ether, ethylacetate and butanol extracts were evaporated to give 105, 55, and 148 g, respectively. The aqueous extract was evaporated on a water bath in a porcelain dish. The solid was dried under vacuum (10 mm Hg, 50-60°C) and ground into a powder. The powder was dried in a vacuum desiccator at 50-60°C and 1 mm Hg. The highly polymerized proanthocyanidins are a light-brown amorphous powder. The yield was 440 g (11% of dry weight).

The ethylacetate extract contains proanthocyanidins consisting of at least nine components giving a positive reaction with vanillin (R_f values 0.09, 0.29, 0.35, 0.37, 0.48, 0.62, 0.69, 0.83, and 0.92 using Systems 1 and 2). The butanol extract contains oligomeric proanthocyanidins consisting of at least 15 components giving a positive reaction for proanthocyanidin.

Separation of Proanthocyanidins. The proanthocyanidins (ethylacetate extract, 50 g) were mixed with cellulose (40 g), placed on a column with cellulose (5×145 cm, 410 g), and eluted with hexane. Fractions (50 ml) 1-40 were collected. Elution with hexane—ethylacetate (1:1, fractions 41-60; 1:3, 61-100; 1:4, 101-190; 1:5, 191-258; 1:10, 259-410; 1:20, 411-450), acetone (451-485), water—acetone (1:20, 486-550) followed. The elutions were monitored by TLC using Systems 2, 3, and 5. Fractions 1-40 contained a mixture of neutral slightly polar oily substances that weighed 5.9 g after removal of solvent.

Rhodichimoside (1). Removal of solvent from fractions 460-473 gave an amorphous light-brown substance: 0.660 g, mp 290-300°C (dec.), $[\alpha]_D^{22}$ -16° (*c* 0.32, ethanol), MW ~2500, R_f 0.09 (System 3). UV spectrum (λ_{max}, nm, ethanol): 220, 245, 278, 301. IR spectrum (ν_{max}, cm^{-1}, KBr): 3500, 1690, 1620, 1545, 1515, 1450, 1320, 1250, 1200, 1045, 830, 805, 774, 730.

Rhodichin (2). Removal of solvent from fractions 490-512 gave an amorphous brown powder: 0.670 g, mp 300°C (dec.), $[\alpha]_D^{22}$ +42° (*c* 0.42, ethanol), MW ~2600, R_f 0.29 (System 3). IR spectrum (ν_{max}, cm^{-1}, KBr): 3500, 1695, 1610, 1540, 1515, 1450, 1320, 1250, 1040, 770, 735.

Alkaline Cleavage of Rhodichin (2). Rhodichin (**2**, 50 mg) was placed in a four-necked round-bottomed 20-ml flask with nitrogen flowing slowly through it. NaOH solution (50%, 5 ml) was added. The lower part of the flask was immersed with constant stirring into a low-melting metal-alloy bath (155-160°C). The bath temperature was increased over 5 min to 230°C. Then the reaction mixture was rapidly cooled by immersing the flask in ice water and acidified with H_2SO_4 (20%). The contents

of the flask were diluted with water and extracted with ethylacetate (50 ml, five times). The ethylacetate extract was dried with anhydrous Na_2SO_4. The solvent was evaporated. The solid was chromatographed on a polyamide column. Two compounds were obtained: **1**, $C_6H_6O_3$, M^+ 126, mp 218-219°C; **2**, $C_7H_6O_5$, M^+ 170, mp 220°C (dec.). The physicochemical and spectral properties (IR, PMR, mass) corresponded to those of phloroglucin and gallic acid.

Alkaline Cleavage of Rhodichimoside (1). This was performed by the method described for rhodichin. Two compounds were obtained and identified as phloroglucin and gallic acid.

Acid Cleavage of Rhodichin (2). Rhodichin (0.08 g) was dissolved in ethanol (2 ml), diluted with HCl (1 ml, 2 N), and heated on a water bath for 2 h with a condenser under nitrogen. The reaction mixture (reddish color) was diluted with water and extracted with ethylacetate (2 ml, three times). The extract was washed with Na_2CO_3 solution and dried over Na_2SO_4. The solvent was evaporated. The solid was chromatographed on a Sephadex LH-20 column (2×120 cm, 200.0 g) using 60% aqueous ethanol. Yield 0.013 g of (-)-epigallocatechin gallate, mp 210-211°C, $[\alpha]_D^{20}$ -135° (*c* 0.06, methanol—water, 1:1), ^{13}C NMR (ppm): 26.4 (C-4), 70.4 (C-3), 78.0 (C-2), 96.1 (C-6), 97.0 (C-8), 99.4 (C-10), 107.2 (C-2′, C-3′), 110.6 (C-2, C-6 galloyl), 121.2 (C-1, galloyl), 130.7 (C-1′), 133.3 (C-4′), 139.5 (C-4, galloyl), 146.9, 146.2 (C-3, C-5 galloyl and ring *B*), 156.6, 156.9, 157.0 (C-5, C-7, C-9 of ring *A*), 167.5 (α-C=O).

Paper chromatography revealed D-glucose (System: *n*-butanol—pyridine—water, 6:4:3, R_f 0.50, anilinium phthalate development) and delphinidin [R_f 0.36 (2N HCl), λ_{max} 554 nm (0.1% HCl in ethanol)] in the hydrolysate of **2**.

Thiolytic Cleavage of Rhodichin (2). Rhodichin (0.088 g) and phenylmercaptan (1.5 ml) were mixed in acetic acid (1 ml) and ethanol (10 ml) and left at room temperature for 10 h. The reaction mixture was condensed. The product was an oily substance that was chromatographed on Sephadex LH-20. Elution with ethanol gave (-)-epigallocatechin gallate (0.009 g) and an amorphous amount of thioethers (0.024 g).

Catalytic Cleavage of Thioethers (11, 12). The thioethers (0.024 g) were mixed with ethanol—acetic acid (1 ml, 9:1). A catalytic amount of Raney nickel was added at 50°C. The temperature was held constant for 30 min. The reaction mixture was filtered. The filtrate was dried and chromatographed on Sephadex LH-20. Elution using 60% aqueous ethanol gave two products (0.005 g, mp 210-212°C; 0.008 g, mp 216-218°C) that were identified as (-)-epigallocatechin gallate (*3*) and (-)-epigallocatechin (**8**).

Thiolytic Cleavage of Rhodichimoside (1). Rhodichimoside (100 mg) was mixed with phenylmercaptan (1.5 ml) and acetic acid (1 ml) in ethanol (10 ml). The mixture was held at room temperature for 16 h and then concentrated. The product was an oily substance that was chromatographed on Sephadex LH-20. Elution using ethanol gave (-)-epigallocatechin gallate (9 mg) and an amorphous thioether (29 mg).

Catalytic Cleavage of Thioether (6). Thioether (29 mg) was mixed with ethanol—acetic acid (1 ml, 9:1). The mixture was treated with a Raney-nickel catalyst at 50°C for 30 min. Then the reaction mixture was filtered. The filtrate was condensed and chromatographed on Sephadex LH-20. Elution using aqueous ethanol (80%) gave a substance (11 mg, mp 210-212°C) that was identified as (-)-epigallocatechin gallate (**3**).

Methylation of 1. The glycoside (0.05 g) was dissolved in DMSO (10 ml), treated with NaH (0.1 g), and stirred at room temperature for 1 h. Methyliodide (5 ml) was added dropwise. The mixture was stirred for another 4 h, poured onto icewater (30 ml), and extracted with $CHCl_3$. The extract was treated with sodium thiosulfate, washed with water, and dried over anhydrous Na_2SO_4. After the solvent was removed the solid was methylated in this way another five times. The products were separated by column chromatography. Yield 0.046 g of amorphous permethylate (**7**).

Hydrolysis of Permethylate of 1. Permethylate (**7**, 0.046 g) was dissolved in aqueous methanol (1:1, 5 ml) containing HCl (5%) and heated on a water bath for 8 h. The reaction mixture was neutralized using $BaCO_3$. The precipitate was filtered off. The filtrate was evaporated to dryness. The solid was purified by column chromatography. Yield 0.016 g of methylated carbohydrates. The methylated sugars were identified by comparing their GLC and TLC behavior with similar samples: 2,3,4,6-tetra-O-methyl-D-glucopyranose, T_{rel} 1.00, 1.46 (phase 1), 1.00, 1.40 (phase 2); 2,3,4-tri-O-methyl-D-glucopyranose, T_{rel} 3.13, 3.73 (phase 1), 1.79, 2.40 (phase 2).

GLC was performed on a Tsvet-4 chromatograph. The methylglycosides that were prepared by boiling the methyl ethers of the sugars in 5% HCl in methanol (4 h) were chromatographed on a column (1 m × 4 mm) containing 20% 1,4-polybutandiol succinate on cellite (phase 1, 160°C thermostatted temperature, He flow rate 5 ml/min) or 10% polyphenyl ester Chromaton N-AW (phase 2, 180°C thermostatted temperature, He flow rate 50 ml/min).

Retention times T_{rel} of the methylated methylglycosides were calculated relative to that of the 2,3,4,6-tetra-O-methyl-β-D-methylglucopyranoside.

TLC of the hydrolysate of the permethylate revealed the presence of the same methylated sugars (system: $CHCl_3$—CH_3OH, 12:1).

Methylation of 2 was performed analogously. The product was 2,3,4,6-tetra-O-methyl-D-glucopyranose.

Enzymatic Hydrolysis of 1 and 2. The glycoside (0.01 g) was dissolved in water (10 ml) and treated with β-glucosidase. The reaction mixture was placed in a thermostatted bath and held for 6 h at 30°C. The polyphenols were precipitated with lead acetate. Paper chromatography of the filtrate revealed D-glucose.

The biological activity of rhodichimoside and rhodichin was studied using male rats (200-220 g mass) and oral administration (50 mg/kg dose).

The effect of rhodichimoside and rhodichin on the lipid level in normal rat serum was studied using either a single dose 2 h before decapitation or during the 10 preceding days. For the hyperlipidemia experiments, rhodichimoside and rhodichin were administered before hyperlipidemia was induced and 2 h before decapitation. Hyperlipidemia was induced by a 24-h fast or by intraperitoneal administration of triton WR-1339 (225 mg/kg). Animals were decapitated after anesthetization with ether after 24 h for the first experiments and after 17 h for the second set. The cholesterol [14] and triglyceride [15] content of the serum was determined as before.

In addition to the rats tested with rhodichimoside and rhodichin, we used a control group and a group that received polysponin (50 mg/kg, oral) as a hypocholesterinemic agent [16].

The anti-inflammatory activity of rhodichimoside and rhodichin was determined in a separate series of experiments that measured the ability to inhibit foot swelling induced by aponeurosis by administering formalin (0.2 ml, 1%), dextran (0.1 ml, 6%), and serotonin (0.5%). The studied compounds were administered to the rats on the evening of the experiment and then 2 h before and 30 min after inoculation with the phlogogenic agents. The results were calculated at the maximum development of foot swelling in the control (determined oncometrically). Some of the animals in these experiments were administered cortisone acetate (10 mg/kg, oral) for comparison. All results were statistically analyzed [17].

REFERENCES

1. E. A. Krasnov, *Khim. Prir. Soedin.*, 545 (1976).
2. Kh. Kh. Kim, Z. A. Kuliev, A. D. Vdovin, M. R. Yagudaev, and V. M. Malikov, *Khim. Prir. Soedin.*, 723 (1989).
3. Kh. Kh. Kim, Z. A. Kuliev, A. D. Vdovin, M. R. Yagudaev, and V. M. Malikov, *Khim. Prir. Soedin.*, 771 (1991).
4. K. N. Matamarova, Z. A. Kuliev, A. D. Vdovin, N. D. Abdullaev, and M. B. Murzubraimov, *Khim. Prir. Soedin.*, 756 (1998).
5. K. N. Matamarova, Z. A. Kuliev, A. D. Vdovin, N. D. Abdullaev, and M. B. Murzubraimov, *Khim. Prir. Soedin.*, 50 (1999).
6. *Flora of the USSR* [in Russian], Academy of Sciences of the USSR, Moscow-Leningrad (1939), Vol. 9, p. 8.
7. *Plant Resources of the USSR. Flowering Plants, Their Chemical Composition, and Use. Caprifoliaceae—Plantoginaceae Family* [in Russian], Nauka, Leningrad (1989), p. 193.
8. A. D. Vdovin, Z. A. Kuliev, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 545 (1997).
9. D. Sun, H. Wong, and L. Y. Foo, *Phytochemistry*, **26**, 1825 (1987).
10. Z. Czehnska, L. Y. Foo, R. H. Newman, and L. I. Porter, *J. Chem. Soc., Perkin Trans. I*, 2278 (1980).
11. F. Hsu, G. Nonaka, and I. Nisioka, *Chem. Pharm. Bull.*, **34**, 3293 (1985).
12. A. G. Kurmukov, S. S. Nazrullaev, and D. G. Abdullakhodzhaeva, Materials of the 2nd All-Union Conference "Pharmacological Treatment of Hypoxic States" [in Russian], Grodno (1991), Part II, p. 245.
13. M. I. Aizikov, Abstract of a Doctoral Dissertation, Tashkent (1993).
14. L. L. Abell, B. B. Levy, B. B. Brodie, et al., *J. Biol. Chem.*, **195**, 357 (1952).
15. B. P. Neri and C. S. Frings, *Clin. Chem.*, **19**, 1201 (1973).
16. M. D. Mashkovskii, *Medicinal Substances* [in Russian], in Two Parts, Part 2, Stereotypnoe Izd. Meditsina, Tashkent (1989), p. 92.
17. M. L. Belen'kii, *Elements of Quantitation of Pharmacologic Effect* [in Russian], Leningrad (1963).